ELSEVIER

Radiotherapy and Oncology 45 (1997) 39–47

RADIOTHERAPY & ONCOLOGY
JOURNAL OF THE EUROPEAN SOCIETY FOR THERAPEUTIC RADIOLOGY AND ONCOLOGY

Is pulsed dose rate more damaging to spinal cord of rats than continuous low dose rate?

Karin Haustermans[a,*], Jack Fowler[a], Willy Landuyt[a], Philippe Lambin[a], Albert van der Kogel[b], Emmanuel van der Schueren[a]

[a]*Experimental Oncology, University Hospital Gasthuisberg, 3000 Leuven, Belgium*
[b]*Institute of Radiotherapy, University Hospital Nijmegen, NL 6500 HB Nijmegen, The Netherlands*

Received 27 May 1997; revised version received 2 September 1997; accepted 4 September 1997

Abstract

Background and purpose: Theoretical calculations suggest that pulsed dose-rate irradiation (PDR) should have approximately the same effectiveness as continuous low dose-rate (CLDR) when the same total dose is given in the same overall time, unless large doses per pulse (>2 Gy) are used and/or non-exponential or very short half-times of repair (<0.5 h) are present in the irradiated tissues. However, few animal experiments have been reported to test this theory, and some of them gave contradictory results. We have carried out experiments to determine whether PDR irradiation of 18 mm of cervical spinal cord in the rat was more or less effective than CLDR at 0.5-1 Gy/h, when the overall average dose rate during each day of PDR was close to the overall CLDR average dose rate.

Materials and methods: PDR was simulated at a within-pulse dose rate of 4 Gy/h by filtered 18 MV X-rays from a linear accelerator. Two PDR schedules were used, 0.69 Gy at 1 h repetition (9 pulses per day) and 2 Gy at 3 h repetition (4 pulses per day), with overnight intervals of 16 and 15 h, respectively. The CLDR was delivered from iridium-192 wires in two concentric rings around a collar designed to fit the necks of rats so that they could eat and drink during the 72 h that was always the duration of the CLDR. Dose rate was then proportional to total CLDR dose. A range of doses was used to obtain dose response-curves, with a 15 Gy top-up dose (at 2 Gy/min, HDR) given on the day after the end of the PDR or CLDR irradiations. Animals were observed for at least 9 months to see whether fore-limb myelopathy developed. A total of 6-8 rats was irradiated per dose point, in two sets of experiments at an interval of 12 months.

Results: A set of 2 Gy fractions (at HDR) given daily, followed by the same top-up dose of 15 Gy at HDR, was available from a previous experiment for planning. Its ED_{50} was 61.2 Gy. The ED_{50} values found for the PDR schedules with 2 Gy at 3 h and 0.69 Gy at 1 h were 59.9 and 60.2 Gy, respectively. These were just 2% more effective than the daily HDR fractions, similar to expectations from theory if two components of repair are present. However, the CLDR irradiations resulted in no myelopathy even after doses up to 68 Gy at 0.94 Gy/h.. Thus PDR over 7 days (not at nights) appears to be more effective than CLDR over 3 days, with an effective dose-modifying factor of at least 1.1 to 1.17.

Discussion and conclusions: Reasons for this absence of effect with CLDR in these experiments are discussed, the most likely explanation being that a substantial component of repair with very short $T_{1/2}$ (<0.5 h) was present in spinal cord of these rats. There is evidence from other experiments elsewhere and in our laboratory for such a fast component of repair. © 1997 Elsevier Science Ireland Ltd.

Keywords: Pulsed dose rate; Two-component repair; PDR; Brachytherapy

1. Introduction

Theoretical predictions and early clinical experience suggest that there is little difference between the iso-effective doses for PDR or LDR, (with the same total dose and overall time), especially for PDR pulses less than about 1 Gy each. However, theoretical predictions [7,13,14,15,28, Sminia et al. pers. commun.] agree that if a tissue has a significant component of repair with a very short half-time, then PDR could cause more biological damage than LDR, especially in late responding tissues. Very short means less than about 0.5 h in this context, particularly when $T_{1/2}$ values approach

* Corresponding author. Department of Experimental Therapy, NKI, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands. Tel.: +31 20 5122036; fax: +31 20 512 2050; e-mail: karinh@hermes.nki.nl

0167-8140/97/$17.00
PII S0167-8140(97)00172-2

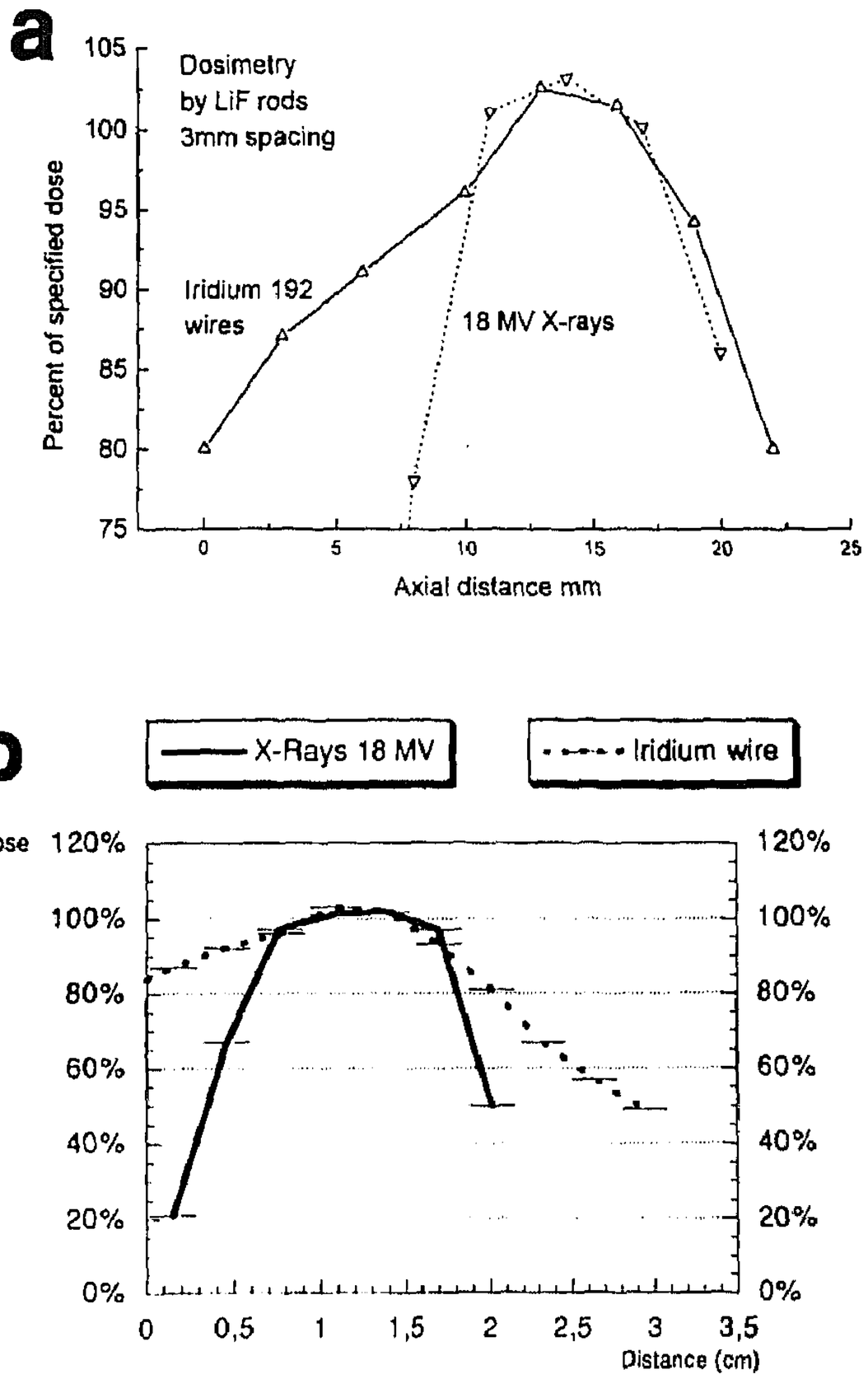

Fig. 1. Results of dosimetry measurements with abutting 3mm long LiF rods along the axis of the spinal cord: two separate determinations.

the duration of the pulses, which can occur with short $T_{1/2}$ if dose rates in the pulse are very high. There has however been no experimental confirmation of this prediction. Experimental animal results comparing PDR with CLDR have mostly given results showing similar effects in a variety of normal tissues for equal doses in the same overall times [5,8,23,27]; but some tumours have demonstrated

Table 1

Pulsed dose rate irradiations at medium dose rate of 4 Gy/h within pulses. Doses quoted do not include the 15 Gy X-ray top-up dose

4 F/day at 3 h intervals (9 h overall/day)		9 F/day at 1 h intervals (8 h overall/day)	
Dose (Gy)	Animals paralysed/ no. at risk	Dose (Gy)	Animals paralysed/ no. at risk
48	0/8	48.30	0/8
52	0/7	51.06	0/6
54	0/8	53.82	0/7
56	0/8	56.58	2/5
58	4/8	58.65	2/5
60	5/8	60.03	4/8
62	4/6	63.48	5/7
66	4/5	66.24	6/7
ED_{50} = 59.91 Gy, (95% CI = 57.82-62.08 Gy)		ED_{50} = 60.22 Gy, (95% CI = 57.64-62.92 Gy)	

Table 2

Daily 2 Gy fractions at 2 Gy/min (as a basis for predicting expected CLDR dose range to use). Doses quoted do not include the 15 Gy X-ray top-up dose

Dose (Gy)	Animals paralysed/ no. at risk	Dose (Gy)	Animals paralysed/ no. at risk
48	0/7	64	4/8
52	0/7	66	7/7
56	1/7	68	7/7
58	0/5	72	6/7
60	8/15	76	8/8
62	4/7		
		ED_{50} = 61.19 Gy, (95% CI = 59.28-63.17 Gy)	

either less [27] or more effects [34] of the PDR. For late rectal injury in rats, Armour et al. [5] showed PDR to become more effective than CLDR only for doses per pulse above 1.5 Gy, in general agreement with theoretical predictions.

The present experiments were carried out, based on our existing system of irradiating the cervical spinal cord of rats, to test whether spinal cord sensitivity to PDR, at a moderate dose rate of 4 Gy/h within the pulse, was the same as for CLDR given at the same average overall-treatment dose rate. Since these experiments were carried out the extant laboratory facilities have ceased to exist, so that further repeats are not possible.

2. Materials and methods

2.1. Animals

Adult male WAG/Rij rats were bred and kept until use in our conventional animal housing facility. Tylosine, a broad spectrum antibiotic for veterinary use, was added to the drinking water to prevent respiratory infections. Irradiations were given at 12-14 weeks of age, determined by weight being close to 270 g.

2.2. Pulsed dose rate and top-up irradiations

These irradiations were done with a linear accelerator producing 18 MV photons. Two blocks of MCP shielding spaced 18 mm apart were placed close above the anaesthetised rats, with 2.5 cm of tissue-equivalent bolus to ensure full electron build-up, as in our previous spinal cord experiments. A semi-closed inhalation anaesthesia system with enflurane and oxygen was used for reproducible positioning [2]. The spinal cord segment of 18 mm length from C2 to T1 was irradiated, as in our earlier experiments [2–4,22]. The dose at these edges of the field was 50% of the central dose (see Fig. 1). A central dose rate of 4.07 Gy/h (100%)was used for the PDR experiments, at a focus-skin distance of 100 cm, the dose rate being reduced only by lowering the

pulse repetition frequency of the linear accelerator. The top-up dose, a single dose of 15 Gy which delivered half of the total effect [3,19], was given at the same distance but at the high dose rate of 2 Gy/min (HDR). The dosimetry was checked at least once each week. The 2 Gy daily irradiations, which were used as the basis of estimations for the dose ranges used, were given at 2 Gy/min and the results are previously published [22], their ED_{50} being 61.2 Gy (95% CI 58.0-64.6 Gy) plus the standard top-up dose of 15 Gy. They were obtained with 8 dose groups of 7-8 rats each. A further set of 4 overlapping dose groups (26 rats) was irradiated with 2 Gy daily doses during the present set of experiments, as a checking control group. When these results were combined with the previous 8 dose groups, the ED_{50} was identical but the CI was somewhat smaller: 61.19 (59.3-63.2) Gy, as shown in Tables 1 and 2.

2.3. Low dose rate irradiations

To assess the response of rat cervical spinal cord to external continuous low dose-rate treatment (CLDR), a new set-up was designed. A specially constructed plastic ring collar was placed around the neck of the rats, nicely fitting between the base of the skull and the scapula. The frame was shaped so as to compensate for the difference in neck thickness over the treatment field and also to allow the animals to eat and drink during the continuous irradiations. A lead shield 3mm thick was taped over the caudal end of the frame to minimise lung doses. Each animal wearing its collar could move around in a cage, which was provided with food and water as usual, during the 72 hours of the continuous irradiation.

Two ^{192}Ir wires were positioned in plastic tubes around the plastic ring, in two circles of diameter 40 mm and 14 mm apart on the same axis. The wires were very well fixed and none came out during the CLDR irradiations. The wires were inserted using long forceps as protection for personnel, together with distance (and speed while fitting and removing the collars). Lead blocks were always placed between the rat cages to prevent additional dose to the animals. With the chosen distance of 14 mm between the two wires, a relatively homogeneous dose distribution over the cervical spinal cord was obtained, with a dose variation of less than ±5% over a 9 mm target length of cord (see Fig. 1). The ^{192}Ir wires were always checked at the Radiation Safety Department of the University Hospital Gasthuisberg, Leuven, for their homogeneity and absolute activity. No discrepancies were found from the measurement of activity at Amersham International, who provided the specially requested wires.

Thermoluminescent dosimetry was performed both in a plastic phantom model and in cadaver rats with the use of lithium fluoride rods. Computer calculated dosimetry was also done using the Villejuif model, with good agreement. Care was taken to irradiate equal lengths of cervical spine with both set-ups. Fig. 1 shows the measured dose distributions along the axis of the spine, using 3 mm long lithium fluoride rods abutting each other. The peaked distribution of the X-ray beam is characteristic of narrow fields and the distribution from the iridium wire circles was flatter as shown. The measurements showed that the lengths of spinal cord irradiated were 7 mm exactly at 97% of the prescribed dose, from both the PDR X-ray beam and the CLDR iridium wire set-ups. At 102% dose the CLDR-irradiated length of spinal cord was possibly up to 2 mm shorter than the PDR set-up (5 vs. 3+ mm) although this difference in Fig. 1 is within experimental variation. At 92% The CLDR field was 4 mm longer, being 13 mm compared with 9 mm for the PDR (see Fig. 1). At all lower dose levels the length irradiated by CLDR was of course greater than that by PDR. This is the evidence that the CLDR irradiations were not given to a shorter length of cord than the PDR irradiations.

This point is very important in view of the results. At doses below 97% of the prescribed doses, the length irradiated was always greater for CLDR than for the sharply collimated PDR irradiations, as shown in Fig. 1. Radiographs of both set-ups taken with control rats showed no movement as great as one spinal vertebra, relative to the shielding. The vertebrae C2 and T1 were regularly seen at the edges of the PDR and HDR set-up. For the LDR animals they were just concealed by the lead collar, at different times in the same rat. Movements of about 1 mm could not be excluded, although they were not seen in the test radiographs. Similar small differences in positioning between the different fractions (pulses) in the PDR set-up are expected.

Another consideration was whether the RBE of ^{192}Ir might differ from that of the 18 MV X-rays used for the PDR, which have a broad distribution of photons peaking at about 7 MeV. The mean photon energy of ^{192}Ir is 0.34 MeV, including 63% of 0.2 MeV and 21.3% 0f 0.46 MeV, so that their RBE was not expected to be lower than that of the higher energy X-rays (43). This point is dealt with further in Section 4.

2.4. Irradiations

The duration of all the CLDR exposures was fixed at 72 h. Each rat wearing the radioactive collar was kept in a cylindrical metal cage, and provided with food and water ad libitum.

The range of CLDR doses, given so as to generate a dose response curve, was achieved with a constant exposure time of 72 h using the same ^{192}Ir wires for a given series, taking into account the radioactive decay. In this way the dose rate changed proportionally to the total CLDR dose throughout each series of irradiations. This would have the effect of steepening the dose-response curve, in a way that was possible to model theoretically. In each of the two CLDR series 3 animals per dose point were irradiated.

The PDR irradiations were done during 8-9 h of each day with overnight gaps but no weekend gaps. The dose rate

within each pulse was 4.07 Gy/h from the linear accelerator. The four 2 Gy pulses at 3 h frequency delivered 8 Gy/day in just over 9 h. The 9 pulses of 0.69 Gy at 1 h frequency delivered 6.21 Gy/day in just over 8 h. The average overall PDR dose-rates during each day were therefore 0.89 and 0.78 Gy/h, respectively, for comparison with the dose-rates for the CLDR irradiations which ranged from 0.55 to 0.94 Gy/h. The 5-7 overnight gaps in the PDR schedules were of course long enough to allow complete repair, unlike the gaps in the daytime PDR, of which there were 19-26 for the 2 Gy pulses and 43-59 for the 0.69 Gy pulses. This was allowed for in our theoretical calculations of PDR effectiveness [9,14,15]. The overall duration of the PDR irradiations was 6 to 8 days, depending on total PDR dose, but the pulses and their daytime gaps occupied 56-72 h, for comparison with the constant 72 h of all the CLDR irradiations. In each of the two PDR series 4 rats per dose point were irradiated.

2.5. *Follow-up after irradiation*

The animals were examined twice per month during the first 5 months and at least weekly for the next 4 months to evaluate movements and reflexes of the forelegs. The animals were always sacrificed when definite signs of foreleg paralysis were seen. This was the biological effect recorded as a myelopathy response, together with its time of onset.

2.6. *Theoretical modelling*

The linear quadratic formula was used [6,9,12] with only the beta term subject to repair at one or more exponential rates. The factor g was computed for the effect of repair during irradiation both for CLDR and for PDR [9,14,15,36]. Calculations were made of Biologically Effective Doses, BED [11], which are numerically identical to ERD (Extrapolated Response Dose, Barendsen [6]), but have a subscript designating the α/β ratio assumed for that calculation. The method of calculating BED or ERD when two (or more) components of repair with different $T_{1/2}$ values are assumed to be present is described in Appendix A.

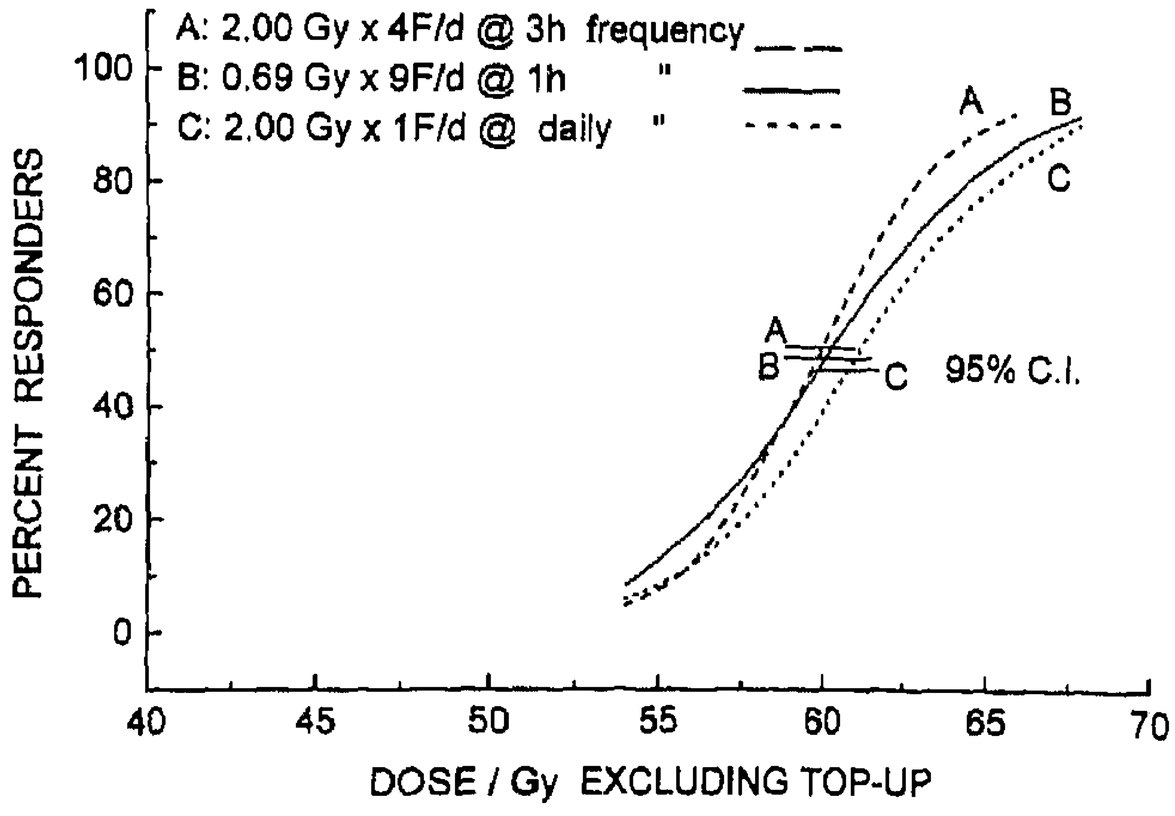

Fig. 2. Spinal cord myelopathy response versus dose curves, for duplicate experiments combined (8 rats per dose point) of the PDR schedules at 4 Gy/h (A and B, Tables 1 and 2) and for an earlier experiment combined with 4 new overlapping dose groups with 2 Gy fractions at 2 Gy/min given daily (C). See Tables 1 and 2. All these irradiations were followed by a 15 Gy top-up dose at 3 Gy/min.

3. Results

Tables 1 and 2 and the upper two curves in Fig. 2 show the results of two series of experiments carried out 12 months apart with the linear accelerator-simulated PDR set-up (4.07 Gy/h) irradiating 4 animals per dose group in each of the series. The two sets of results were closely similar and are combined (for each PDR schedule separately) in Tables 1 and 2 and Fig. 2. Where less than 8 rats are shown at risk in the table, the others in that dose group died from oesophageal toxicity without showing signs of paralysis before the end of the experiment, which was at least 9 months after the end of irradiation. The lowest curve in Fig. 2 is for 2 Gy fractions given daily (at 2 Gy/min), a result obtained earlier and already published [22], which was used as a basis for selecting the range of doses applied in the present series. Neither the single-dose ED50 nor the 2 Gy HDR daily ED_{50} had changed over this period.

It can be seen in Fig. 2 that both the 2 Gy and 0.69 Gy pulsed schedules were about 2% percent more effective than the 2 Gy HDR fractions given at daily intervals (±5% CI), none being significantly different from the others. This is just as predicted for both pulsed schedules at 4 Gy/h, and the daily 2 Gy fractions, provided that two half-times of repair are present, for example approximately 20-30% of $T_{1/2}$ = 3-5 h and 70-80% of $T_{1/2}$ = 0.25 h (methods of Dale [9], Fowler and Mount [14], and Fowler and Van Limbergen [15], see Appendix A). Similar half-times to these were indeed found in earlier experiments on rat cervical spinal cord from this laboratory [22].

Fig. 3 shows the same two PDR curves, with their data points, and in addition all the data points from the two CLDR series (the second being a repeat of the first with higher dose groups up to 76 Gy, irradiated 1 year after the first, when an extended follow-up period had shown no responders up to doses of 60 Gy at 0.83 Gy/h). In the second CLDR series, no animals survived 70 Gy or more. The highest dose group with any rats surviving received 68 Gy, in which 2 of the 4 rats died within 1 month after irradiation. The animals which died in the irradiated groups did so either a few days after the end of irradiation, or at most within 1 month. Their death was accompanied by severe weight loss, and on the basis of post-mortem observations was attributed to acute oesophagitis leading to starvation and dehydration.

The absence of any responders in both of the CLDR series is clear in Fig. 3. The increasing proportion of acute death in the highest dose groups, and also the dose-related skin damage, with moist desquamation and with hair loss that

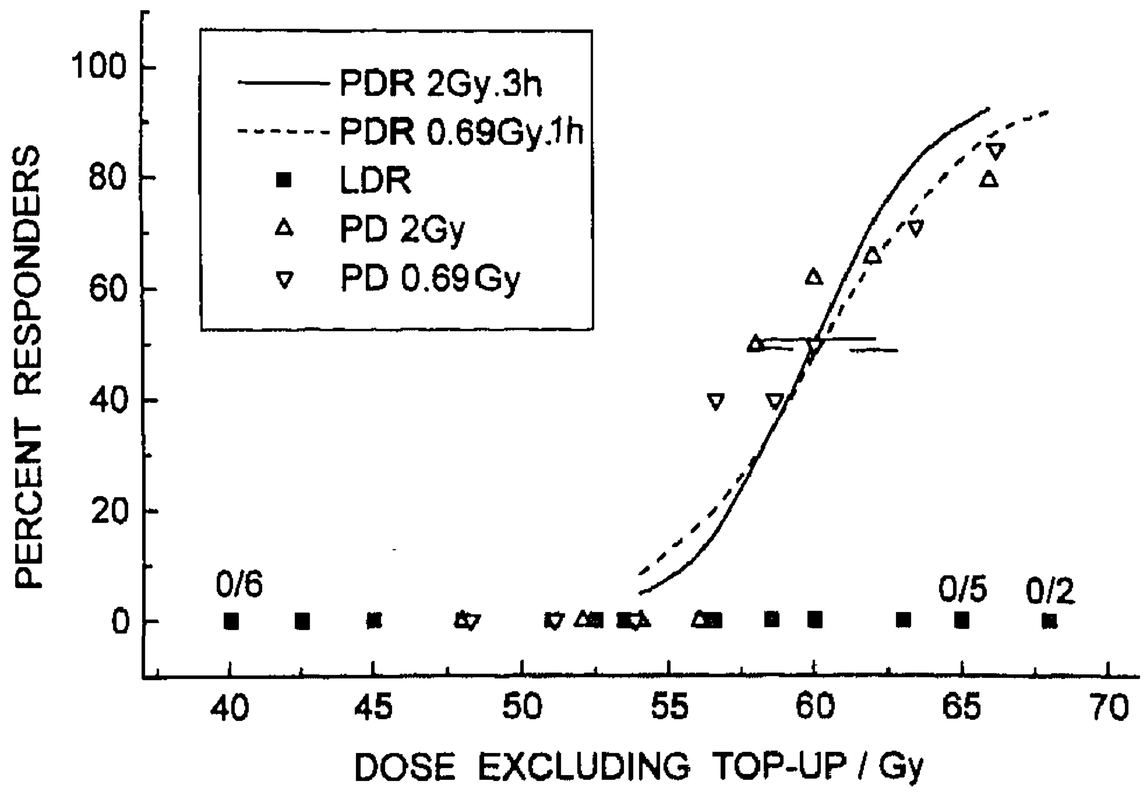

Fig. 3. Data points and curves for the two PDR schedules and data points for the duplicate CLDR experiments. The CLDR exposures were all given with the same duration of 72 hours.

continued throughout the follow-up period, confirm that irradiation was undoubtedly being given. In addition, the dose calibration measurements were as they should be for all the checks. Fig. 4 and Table 4 show the single-dose results (without top-up) obtained contemporaneously with the present PDR and CLDR experiments, together with the 2 Gy daily fractions at 2 Gy/h from the previous experiment which were available to help plan the present irradiations (Tables 1 and 2).

The obvious interpretation from Fig. 3 is that the ED_{50} for the CLDR up to 0.94 Gy/h was more than 68 Gy. The 95% confidence intervals of ED50 in Fig. 2 (when transposed graphically from horizontal to vertical) spread only as wide as from 20-25 to 70-80% response for this system, so by analogy the ED_{50} for the nil response at CLDR should be significantly higher than 68 Gy. More precisely, the two highest CLDR dose groups combined contained 7 animals surviving for the full follow-up time without response (Table 3). The probability of this occurring by pure chance is one in $2^7 = 1/128 = 0.008$ by binomial statistics, and

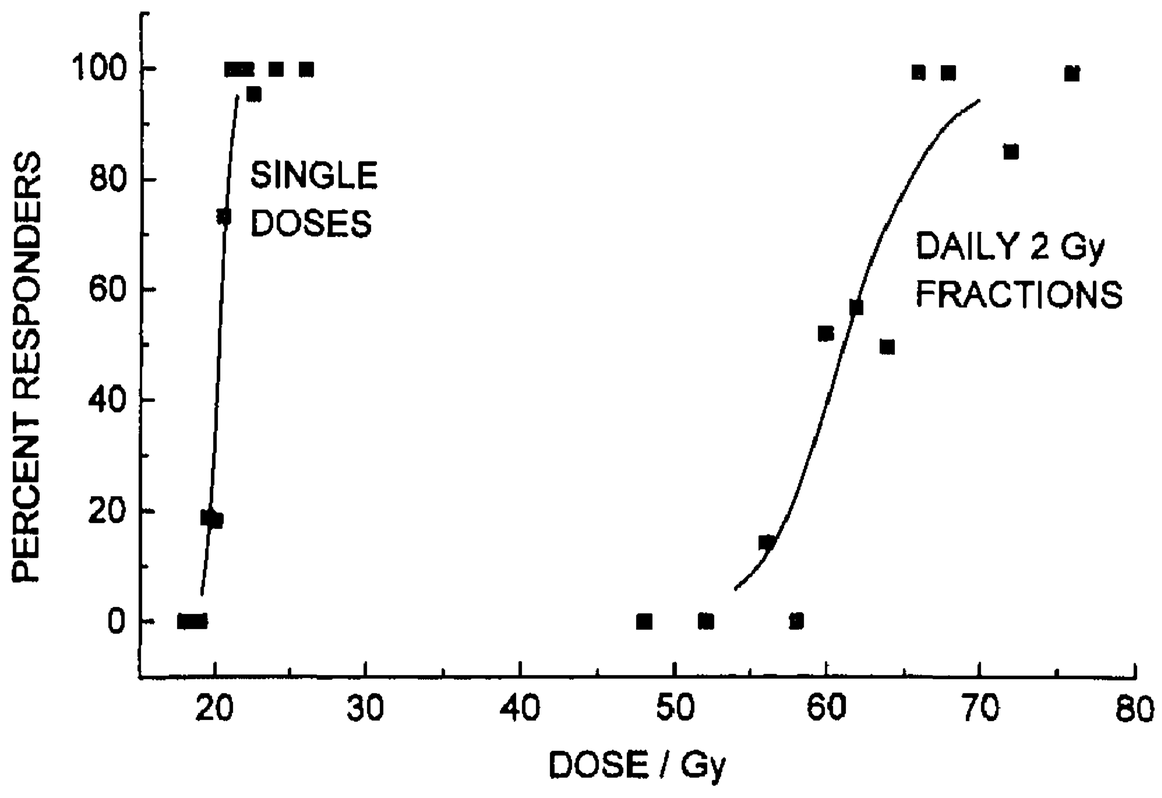

Fig. 4. Data points and curves for the contemporaneous single dose irradiations, without top-up, and for the earlier experiment with 2 Gy fractions given daily plus a 15 Gy top-up dose. These irradiations were at 2 Gy/min.

Table 3

Continuous low dose rate irradiations, all in 72 h. Dose rate proportional to total dose, from 0.94 Gy/h for 68 Gy at start to 0.54 Gy/h for 40 Gy at end of a series. Doses quoted do not include the 15 Gy X-ray top-up dose

Dose (Gy)	Animals paralysed/ no. at risk	Dose (Gy)	Animals paralysed/ no. at risk
40	0/6	56.1	0/3
42.5	0/6	56.5	0/5
45	0/4	58.5	0/3
48	0/4	60	0/3
51	0/3	63	0/3
52.5	0/2	65	0/5
53.5	0/1	68	0/2

No paralysis at any dose up to and including 68Gy, even when follow-up period extended from 9 to 11 months. $ED_{50} > 68.0$Gy, or combining the two highest-dose groups > 65.9 Gy.

lower by Poisson statistics. This means that the probability of zero response by chance in those two dose groups is $P < 0.05$. Therefore the ED_{50} of the CLDR can be regarded as significantly higher than the weighted average of 65 and 68 Gy (5 and 2 animals, respectively), which is 65.9 Gy.

If we take, conservatively, the highest dose at which no response was observed as 65.9 Gy, and if we assume that a CLDR response curve should have a similar slope to the PDR or daily HDR curves, then the ED_{50} value for CLDR might have been above 70 Gy, but was certainly above 65.9 Gy. These values are to be compared with the ED_{50} values of 59.9 and 60.2 Gy for the PDR schedules, which average 60 Gy.

Therefore the dose modifying ratio suggested by these experiments for the PDR schedules compared with the CLDR was most likely to be 65.9/60 = 1.10 or even 70/60 = 1.17.

4. Discussion

The dose-response curves obtained were all technically good, with acceptable 95% confidence intervals on the ED_{50} (and other damage level) values, as shown by the graphs. However, with the CLDR irradiations no myelopathy response at all was seen, up to the highest doses at which the animals survived.

Table 4

Linac high dose rate (2 Gy/min) single doses. No top-up doses were given

Dose (Gy)	Animals paralysed/ no. at risk	Dose (Gy)	Animals paralysed/ no. at risk
18	0/12	21.5	16/16
19	0/6	22	8/8
19.5	3/16	22.5	21/22
20	2/11	24	16/16
20.5	11/15	26	8/8
21	5/5		
		ED_{50} = 20.22 Gy, (95% CI = 19.93-20.51 Gy)	

There could be four possible explanations for this nil result:

1. *There was no irradiation given, or very much less dose than expected*? However, the acute deaths in the highest dose groups, and the skin reactions which were strongest at the highest doses, including prolonged epilation, and the absence of any peculiar dosimetry readings, all provide convincing evidence against this. None of the ^{192}Ir wires fell out of the CLDR collars or was displaced.
2. *The length of spinal cord irradiated at LDR was significantly less than at PDR*? Coupled with a significant variation of ED_{50} with length of cord irradiated around 7 mm. The possible increase of ED_{50} with decreasing length irradiated in the rat spinal cord has been reviewed thoroughly by van der Kogel [40]. It is true that ED_{50} appears to rise for lengths of cord just less than 7 mm, (Fig. 1 in Ref. [40]). Although the increase is small for van der Kogel's own data, it is larger for the data of Hopewell et al [18], which could be a problem for the present experiments if the length irradiated had been less for the CLDR than for the PDR set-ups. But as shown in Fig. 1, the reverse is true except for the peak dose region encompassing the top 3% of dose, above the specified 100% level.
3. *There was possible movement of the rats' necks within the CLDR holding devices*? So that the dose was spread over a larger volume than planned, resulting in a lower average dose than if stationary. The axial restriction of movement was very good, both by observation of rats wearing the collar and by checking radiographs, so that the lengths shown in Fig. 1 should indeed have been irradiated precisely, at least within ±1 mm. Radial movement would have been less likely, and less important because the radial dose distribution across the centre of the two wire rings was rather uniform. Also, the repeated irradiations of the anaesthetised rats in the PDR groups would involve similar variability of about ±1 mm in longitudinal position.
4. *The RBE of the PDR (18 MV X-rays) was unexpectedly greater than the RBE of the ^{192}Ir wires*? This is a priori most unlikely because the LET and Lineal Energy y_D of electrons secondary to photons decreases with their increasing energy when all the photons are in the energy range of predominantly Compton interactions as here; and RBE increases with LET, at least up to about 100 keV/μm, which is far above the relevant range here. Table 5 shows that this trend is maintained from the photon energy of ^{192}Ir up to 42 MV photons, as expected from first principles [21]. Further, microdosimetric measurements of several nuclides used in brachytherapy have yielded estimates of RBE of 1.3 for ^{192}Ir (0.34 MeV photons) relative to 1.0 for ^{60}Co (1.25 MeV); this is the same trend [43]. The generation of positron pairs by 18 MV X-rays would contribute photons of 0.51 MeV. These too are more energetic than the photons of ^{192}Ir (all of them undergoing predominantly Compton interactions). It is not obvious how these could lead to a greater RBE for the 18 MV X-rays than for the iridium. Thus there is no evidence for a greater RBE of the radiation used for the PDR here, which is needed to explain the observations; indeed the opposite is more likely to be true.
5. *There were two components of repair present, including a substantial component of repair shorter than 0.15-0.5 h, in the spinal cord of these rats*? Several other publications have demonstrated biexponential repair with both a short and long $T_{1/2}$ in the spinal cord of rats [1,22,25,26,28,29,32]. These include one set of experiments from our group using the same strain of rats and the same HDR irradiation set-up as in the present experiments [22].

We have given reasons in Sections 2 and 3 and just above why the first four explanations are unlikely. Finally, we do not consider that the longer length of spinal cord irradiated simultaneously by the ^{192}Ir wires than by the PDR X-rays is likely to diminish the likelihood of myelitis in the target section.

We consider that the fifth explanation is not only the most likely one, but is well supported by other research evidence for a fast component of repair in spinal cord of rats of this and closely associated strains [22,29,31,32] as well as of other strains [1,20]. Other tissues too have been reported to have a short and a long component of repair, including human telangiectasia [38], human oropharyngeal mucosa and skin [10], mouse lung [16,41], pig skin [26,39], rat kidney cells [24] and mouse lip mucosa [35].

This explanation of the absence of response is illustrated by the modelling calculations shown in Fig. 5. For clarity only one monoexponential component of repair is assumed for Fig. 5; the bi-exponential situation is illustrated in Appendix A. As the assumed $T_{1/2}$ of repair is decreased, the biologically effective dose (BED) for the CLDR irradiation also decreases, linearly with $T_{1/2}$. If this BED is less than that of the foot of the response curve of the 2 Gy daily schedule, (which can be regarded as 56 Gy for the 2 Gy fractions and the two PDR schedules tested, see Fig. 2), then we should not expect the CLDR schedule to be effective enough to cause any responses. This situation is illustrated by the crossing of the CLDR and the horizontal curves in Fig. 5. The values of $T_{1/2}$ below which no response is expected were both about 0.55 h whether α/β was 2 Gy or 1.8 Gy, as shown by the vertical lines in the Fig. 5. A substantial component of repair with $T_{1/2}$ of 0.55 h or less would therefore be expected to yield the results observed. Precise modelling can be carried out for mixtures of two (or more) components, each of monoexponential repair, by the procedure described in .

It is noteworthy that a short component of $T_{1/2} = 0.25$ (95% CI 0.16-0.48) h has indeed been found in the same strain of rats by Direct Analysis [37] of data from other

Table 5
Lineal Energy and LET values for photons and electrons.

MeV *A: Photon Energy*	keV/μm (1 micron site diameter) *Measured lineal energy, y_D. [21]*
0.33	1.00
0.66	0.77
1.25	0.67
42	0.57
B: Electron Energy	*Restricted $LET_{100\ eV}$*
0.2	0.17
0.5	0.12
1.0	0.11
2.0	0.10
5.0	0.10
10.0	0.10

Lower LET or y_D is associated with lower RBE, or unchanged RBE.

experiments in this laboratory [22]. Other laboratories also report two components of repair in spinal cord of rats [1,20,29,31,32]. There is therefore no conflict between the modelled and the presently observed results, which were of nil response to CDLR up to at least 65.9 or 70 Gy. The resulting dose modifying factors are at least 1.10 or 1.17 respectively.

5. Conclusions

The absence of any response in the CLDR irradiated rats suggests that CLDR up to 0.94 Gy/h was less effective than PDR at 'overall dose rates during the treatment day' up to 0.89 Gy/h (e.g. 0.69 Gy/pulse every hour for 8 h, or 2 Gy/pulse every 3 h for 9 h), by a dose-modifying factor of at least 1.1-1.17. This was so even though the overall time of the CLDR was 3 days and that of the PDR schedules was 7 days, given during 8-10 h each day, which would have given less opportunity for any 'slow repair' in the CLDR groups. It should be noted that dose rate within the pulse is less important than dose per pulse in PDR irradiations [6,13,14,26]. From theoretical calculations these DMFs can easily be explained by a substantial component of short $T_{1/2}$ of repair of sublethal radiation injury (the beta component). Other results, as referenced above, demonstrating that a rapid component of repair is present in the spinal cord of rats, make this a most likely explanation. No quantitative estimate of $T_{1/2}$ can be obtained because there were no responders in the CLDR groups.

If the present lack of response in the CLDR arm is validly explained as due to both a fast and slow component of repair, it raises a warning flag for any irradiations using pulsed scheduling, whether external beams or brachytherapy, where the central nervous system could be the tissue at risk. We are unable to comment on the possibility that peripheral nerves might respond in a similar fashion, i.e. that PDR might be at least 10-17% more damaging than CLDR with the same total dose and overall time. Further, we do not know half-times of repair in human spinal cord for comparison with the data from rats, but the explanation of the four myelopathy cases reported in the CHART pilot study [1,11,17,33,40,42] would obviously become easier if there were two components of repair, one with a longer and one with a shorter $T_{1/2}$ than previously thought.

The present result should not be generalised too widely to other normal tissues. It is known that some other tissues might have two components of repair [16,17,25,26,39,41], including human tissues [10,38]. In view of this possibility, and also in view of some early clinical results which showed telangiectasia at earlier times or unexpected necrosis with PDR treatments [30], the previous radiobiological warning to keep within the cautious envelope of small doses per pulse should not be forgotten [7,14].

Acknowledgements

We have pleasure in thanking Monique Ramaekers for her skilled and dedicated assistance in managing the animals and carrying out the irradiations and subsequent follow-up. We thank also Ing. Alex Rijnders for his help with the dosimetry of both the X-ray and the iridium-192 wire set-ups, Professor Andree Dutreix for advice on the calibration and dose calculations for the iridium wires, and Dr. Erik Van Limbergen for discussions concerning relevance to the clinic. We are grateful to Drs. David Brenner and Cliff Ling for advice on the microdosimetry and RBE of ^{192}Ir. The Gray Laboratory and the Medical Illustration Department at Mount Vernon Hospital, Northwood, generously contributed computational and graphical facilities.

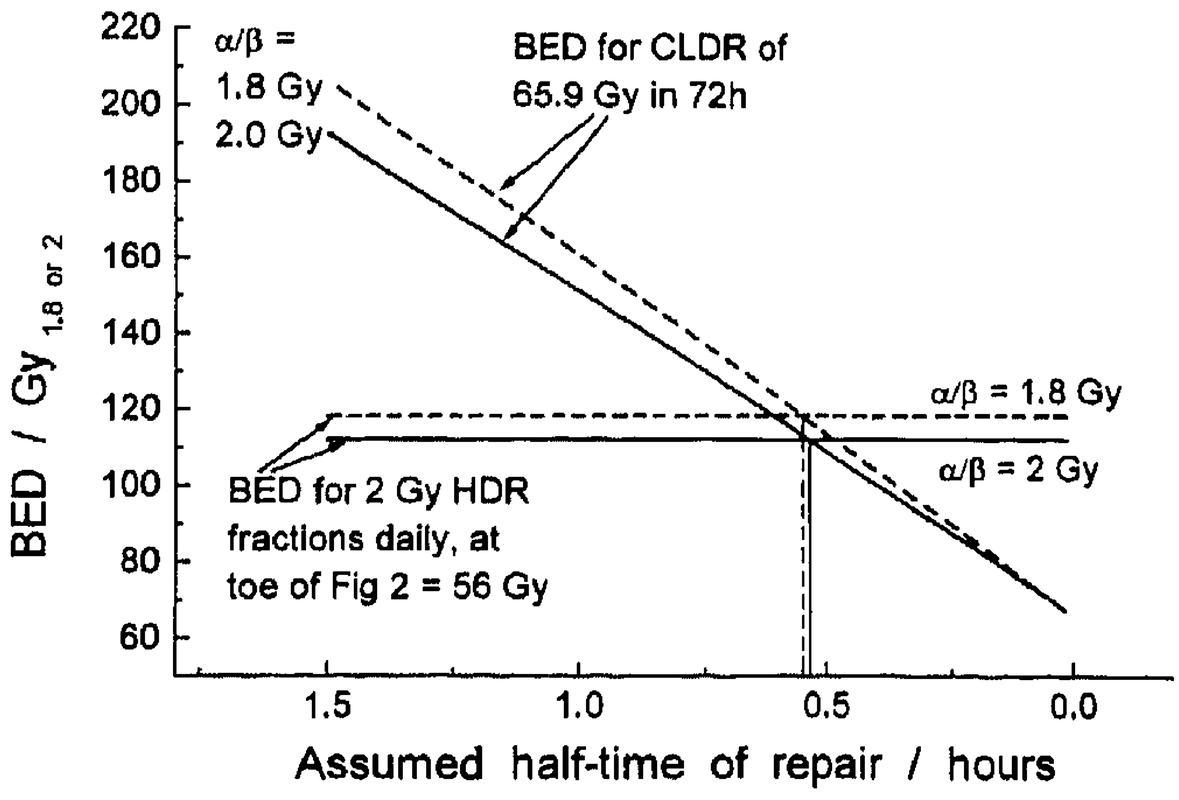

Fig. 5. Model calculations illustrating the decrease of Biologically Effective Dose of the highest CLDR data point with shorter assumed $T_{1/2}$ of repair, for two assumed ratios of α/β. 1.8 Gy (dashed) and 2 Gy (full line). The horizontal lines represent the BED for the 2 Gy fractions given daily and the two PDR schedules, at 56 Gy, i.e. just above the threshold of response, from Fig. 2. The point where each pair of lines for a given α/β value crosses indicates (vertical lines) the $T_{1/2}$ value which must be exceeded for any response to be expected from this CLDR exposure. For simplicity monoexponential repair is assumed in this diagram. For two components see text and Appendix A.

Appendix A. Radiobiological effect of two (or more) components of repair

The radiobiological effect of two (or more) components of repair, of different monoexponential half-times of repair, can be calculated by the following procedure. The BED or RE for the total dose given in the actual overall time, with the actual time configuration (LDR, PDR or HDR) should be calculated separately for each putative value of $T_{1/2}$. These BEDs (or REs if calculated for the correct total doses) should finally be added in the appropriate proportions of each assumed $T_{1/2}$.

Following this procedure to investigate the similar ED_{50} values of the three schedules in Fig. 2 resulted in the following conclusions, assuming $\alpha/\beta = 2$ Gy for this example:

RE for 2Gy × 4F at 4Gy/h PDR in 1 day with 3 h frequency if $T_{1/2}$ is 0.25 h = 1.663.
RE for 2 Gy × 4F at 4Gy/h PDR in 1 day with 3 h frequency if $T_{1/2}$ is 5.0 h = 3.456.
What mixture of these would give the same RE as 2 Gy fractions at HDR daily?
Now the RE for 2Gy at 2 Gy/min with 24 h frequency = 1 + 2/2 = 2.00.

Let Q be the proportion of the slower component, 1-Q that of the faster. Then, for equal effect (same ED_{50}) the REs must be equal:

$$\text{So} \quad 3.456Q + 1.663\,(1-Q) = 2.00,$$

from which we get $Q = 0.18$, so the proportions are 18 and 82% for equality.

If we allow that the CIs are wide enough to conceal a 5% difference in Fig. 2 (although not statistically significant), then the proportions could be 22% and 78% (or 14 and 86%) for the slower and faster components respectively. This method is the source of the ratios of possible $T_{1/2}$ values quoted in Section 3.

References

[1] Ang, K.K., Jiang, G.L., Guttenberger, R., Thames, H.D., Stephens, L.C., Smith, C.D. and Feng, Y. Impact of spinal cord repair kinetics on the practice of altered fractionation schedules. Radiother. Oncol. 25: 287-294, 1992.

[2] Ang, K.K., van der Kogel, A.J. and van der Schueren, E. Inhalation anesthesia in experimental radiotherapy: a reliable and time-saving system for multifraction studies in a clinical department. Int. J. Radiat. Oncol. Biol. Phys. 8: 145-148, 1982.

[3] Ang, K.K., van der Kogel, A.J. and van der Schueren, E. The effect of small radiation doses on the rat spinal cord: the concept of partial tolerance. Int. J. Radiat. Oncol. Biol. Phys. 9: 1487-1491, 1983.

[4] Ang, K.K., van der Kogel, A.J., van Dam, J. and van der Schueren, E. The kinetics of repair of sublethal damage in the rat cervical cord during fractionated irradiation. Radiother. Oncol. 1: 247-253, 1984.

[5] Armour, E.P., White, J.R., Armin, A.R., Corry, P.M., Coffey, M., DeWitt, C. and Martinez, A.A. Pulsed low dose rate brachytherapy in a rat model; dependence of late rectal injury on radiation pulse size. Int. J. Radiat. Oncol. Biol. Phys. 38: 824-835, 1997.

[6] Barendsen, G.W. Dose fractionation, dose rate and iso-effect relationships for normal tissue response. Int. J. Radiat. Oncol. Biol. Phys. 8: 1981-1997, 1982.

[7] Brenner, D.J., and Hall, E.J. Conditions for the equivalence of continuous to pulsed low dose rate brachytherapy. Int. J. Radiat. Oncol. Biol. Phys. 20: 181-190, 1991.

[8] Brenner, D.J., Hall, E.J., Randers-Pehrson, G., Huang, Y.P., Jonnson, G.W., Miller, R.W., Wu, B., Vasquez, M.E., Medvedovsky, C. and Worgui, B.V. Quantitative comparisons of continuous and pulsed dose-rate regimens in a model late-effect system. Int. J. Radiat. Oncol. Biol. Phys. 34: 905-910, 1996.

[9] Dale, R.G. The application of the linear-quadratic model to fractionated radiotherapy when there is incomplete normal tissue recovery between fractions, and possible implications for treatments involving multiple fractions per day. Br. J. Radiol. 59: 919-927, 1986.

[10] Denham, J.W., Hamilton, C.S., Simpson, S.A., O'Brien, M.Y. and Ostwald, P.M. Acute reaction parameters for human oropharyngeal mucosa. Radiother. Oncol. 35: 129-137, 1995.

[11] Dische, S. and Saunders, M.I. Continuous, hyperfractionated accelerated radiotherapy (CHART): an interim report upon late morbidity. Radiother. Oncol. 16: 65-72, 1989.

[12] Fowler, J.F. The linear quadratic formula and progress in fractionated radiotherapy. A review. Br. J. Radiol. 62: 679-694, 1989.

[13] Fowler, J.F. Why shorter half-times of repair lead to greater damage in pulsed brachytherapy. Int. J. Radiat. Oncol. Biol. Phys. 26: 353-356, 1993.

[14] Fowler, J.F. and Mount, M. Pulsed brachytherapy: The conditions for no significant loss of therapeutic ratio compared with traditional low dose rate radiotherapy. Int. J. Radiat. Oncol. Biol. Phys. 23: 661-669, 1992.

[15] Fowler, J.F. and Van Limbergen, E. Biological effect of pulsed dose rate brachytherapy with stepping sources if short half-times are present in tissues. Int. J. Radiat. Oncol. Biol. Phys. 37: 877–883, 1997.

[16] Fowler, J.F., Whitsed, C.A. and Joiner, M.C. Repair kinetics in mouse lung: a fast component at 1.1 Gy per fraction. Int. J. Radiat. Biol. 56: 335-353, 1989.

[17] Guttenberger, R., Thames, H.D. and Ang, K.K. Is the experience with CHART compatible with experimental data? A new model of repair kinetics and computer simulations. Radiother. Oncol. 25: 280-286, 1992.

[18] Hopewell, J.W., Morris, A.D. and Dixon-Brown, A. the influence of field size on the late tolerance of the rat spinal cord to single doses of X rays. Br. J. Radiol. 60: 1099-1108, 1987.

[19] Joiner, M.C. The design and interpretation of 'top-up' experiments to investigate the effects of low radiation doses. Int. J. Radiat. Biol. 51: 115-130, 1987.

[20] Kim, J.J., Hao, Y., Jang, D. and Wong, C. S. Lack of influence of sequence of top-up doses on repair kinetics in rat spinal cord. Radiother. Oncol. 43: 211–217, 1997.

[21] Kliauga, P. and Dvorak, A. Microdosimetric measurements of ionisation by monoenergetic photons. Radiat. Res. 73: 1-20, 1978.

[22] Landuyt, W., Fowler, J., Ruifrol, A., Stuben, G., van der Kogel, A.J. and van der Schueren, E. Kinetics of repair in the spinal cord of the rat. Radiother. Oncol. 1997 (in press).

[23] Mason, K.A., Thames, H.D., Ochran, T.G., Ruifrok, A.C.C. and Janjan, N. Comparison of continuous and pulsed low dose rate brachytherapy: Biological equivalence in vivo. Int. J. Radiat. Oncol. Biol. Phys. 28: 667-671, 1994.

[24] Millar, W.T. and Canney, P.A. Derivation and application of equations describing the effects of fractionated protracted irradiation, based on multiple and incomplete repair processes. Part 2. Analysis of mouse lung data. Int. J. Radiat. Biol. 64: 293-303, 1993.

[25] Millar, W.T., Jen, J.M., Hendry, J.H. and Canney, P.A. Two component repair in irradiated kidney colony forming cells. Int. J. Radiat. Biol. 66: 189-196, 1994.

[26] Millar, W.T., van den Aardweg, G.J.M.J., Hopewell, J.W. and Canney, P.A. repair kinetics in pig epidermis - an analysis based on two separate rates of repair. Int. J. Radiat. Biol. 69: 123-140, 1996.
[27] Moulder, J.E. and Fish, B.L. The equivalence of pulsed and continuous low dose rate in normal tissue models. Abstract P15-279. Program and Abstracts of the 44th Annu. Mtg. Rad. Res. Soc., April 1996. p. 144, 1996.
[28] Pop, L.A.M., van den Broek, J.F.C.M., Visser, A.G. and van der Kogel, A.J. Constraints on the use of repair half times and mathematical modelling for the clinical application of HDR and PDR treatment schedules as an alternative for LDR brachytherapy. Radiother. Oncol. 38: 153-162, 1996.
[29] Pop, L.A.M., van der Plas, M., Ruifrok, A.C.C., Hanssen, E.A.J. and van der Kogel, A.J. Tolerance of the rat spinal cord to continuous interstitial irradiation. Int. J. Radiat. Oncol. Oncol. Biol. Phys. (submitted Feb) 1997.
[30] Roed, H., Engelholm, S.A., Svendsen, L.B, Rosendal, F. and Olsen, K.J. Pulsed dose rate (PDR) brachytherapy of anal carcinoma. Radiother. Oncol. 41: 141-134, 1996.
[31] Ruifrok, A.C.C., Kleiboer, B.J. and van der Kogel, A.J. Fractionation sensitivity of rat cervical spinal cord during radiation retreatment. Radiother. Oncol. 25: 295-300, 1992.
[32] Ruifrok, A.C.C., Kleiboer, B.J. and van der Kogel, A.J. Repair kinetics of radiation damage in the developing rat cervical spinal cord. Int. J. Radiat. Biol. 63: 501-508, 1993.
[33] Saunders, M.I., Dische, S., Grosch, E.J., Fermont, D.C., Ashford, R.F.U., Maher, E.J. and Makepeace, A.R. Experience with CHART. In. J. Radiat. Oncol. Biol. Phys. 21: 871-878, 1991.
[34] Sethi, T., Dixon, B., Flynn, A. and Ash, D. Continuous, pulsed or single acute irradiation of a transplanted rodent tumour model. Radiother. Oncol. 43: 203-209, 1997.
[35] Stuben, G., van der Kogel, A.J. and van der Schueren, E. Biological equivalence of low dose rate to multifractionated high dose rate irradiations: investigations in mouse lip mucosa. Radiother. Oncol. 42: 189-196, 1997.
[36] Thames, H.D. An 'incomplete-repair' model for survival after fractionated and continuous irradiations. Int. J. Radiat. Biol. 47: 319-339, 1985.
[37] Thames, H.D., Rozell, M.E., Tucker, S. L., Ang, K.K., Fisher, D.R. and Travis, E.L. Direct analysis of quantal response data. Int. J. Radiat. Biol. 49: 999-1009, 1986.
[38] Turesson, I. And Thames, H.D. Repair capacity and kinetics of human skin during fractionated radiotherapy: erythema, desquamation, and telangiectasia after 3 and 5 years' follow-up. Radiother. Oncol. 15: 169-188, 1989.
[39] van den Aardweg, G.J.M.J. and Hopewell, J.W. The kinetics of repair for radiation-induced sublethal damage in the pig epidermis: an interpretation based on a fast and a slow component of repair. Radiother. Oncol. 23: 94–104, 1992.
[40] Van der Kogel, A.J. Dose-volume effects in the spinal cord. Radiother. Oncol. 29: 105-109, 1993.
[41] van Rongen, E., Thames, H.D. and Travis, E.L. Recovery from radiation damage in mouse lung: interpretation in terms of two rates of repair. Radiat. Res. 133: 225-233, 1993.
[42] Wong, C.S., Van Dyk, J. and Hill, R.P. Myelopathy and hyperfractionated accelerated radiotherapy: A radiobiologic interpretation. Recent Results Cancer Res. 130: 189-197, 1993.
[43] Wuu, C.S., Kliauga, P., Zaider, M. and Amols H.I. Microdosimetric evaluation of relative biological effectiveness for ^{103}Pd, ^{125}I, ^{241}Am, and ^{192}Ir brachytherapy sources. Int. J. Radiat. Oncol. Biol. Phys. 36: 689-697, 1996.